PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 5/06, 5/08, C07D 503/00, A61K 38/55, 31/395 — A1

(11) International Publication Number: WO 97/38008

(43) International Publication Date: 16 October 1997 (16.10.97)

(21) International Application Number: PCT/IB97/00382

(22) International Filing Date: 9 April 1997 (09.04.97)

(30) Priority Data:
60/015,288 10 April 1996 (10.04.96) US

(71) Applicants: SYNPHAR LABORATORIES INC. [CA/CA]; 2 Taiho Alberta Center, 4290-91A Street, Edmonton, Alberta T6E 5V2 (CA). NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; EG12, M-58 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).

(72) Inventors: SINGH, Rajeshwar; 7927-22 Avenue, Edmonton, Alberta T6K 1Z2 (CA). ZHOU, Nian, E.; 425 Michener Park, Edmonton, Alberta T6H 4M5 (CA). GUO, Deqi; 1502 GH, Michener Park, Edmonton, Alberta T6H 5B5 (CA). KALETA, Jadwiga; 3735-60 Street, Edmonton, Alberta T6L 1V4 (CA). CAMERON, Alan; 2 Taiho Alberta Center, 4290-91A Street, Edmonton, Alberta T6E 5V2 (CA). PURISIMA, Enrico; 4910 Geneviève, Pierrefonds, Montreal, Quebec H9J 1S5 (CA). MENARD, Robert; 2702 Modugno Apartment #1, St. Laurent, Quebec H4R 1Z9 (CA). MICETICH, Ronald, George; 12 Braeside Terrace, Sherwood Park, Alberta T8A 3V6 (CA).

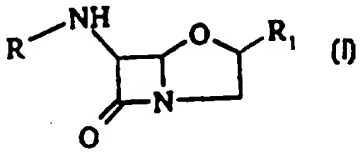
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: 6-SUBSTITUTED AMINO-4-OXA-1-AZABICYCLO[3,2,0] HEPTAN-7-ONE DERIVATIVES AS CYSTEINE PROTEASE INHIBITORS

(57) Abstract

Novel 6-substituted amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one compounds, as well as the pharmaceutically acceptable salts thereof and diastereoisomers thereof, of formula (I) are disclosed, which exhibit excellent cysteine protease inhibitory activity and my be used for treatment of different diseases such as muscular dystrophy, arthritis, myocardial infarction, Alzheimer's disease, bacterial infection, common cold, osteroporosis and cancer metastasis.



(I)

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

6-Substituted amino- 4-Oxa-1-Azabicyclo [3,2,0] Heptan-7-One Derivatives as Cysteine Protease Inhibitors

Background of invention

Cysteine proteases, such as cathepsins B, L, S, and O_2, have been implicated in a number of diseases, including cancer metastasis and invasion (Clin. Exp. Metastasis 1992, 10, 145-155; Cancer Metastasis Rev. 1990, 9, 333-352), arthritis (Int. J. Biochem. 1993, 25, 545-550; Arthritis Rheumatism 1994, 37, 236-247; J Rheumatol. 1993, 20, 1176-1183; Biochem. Pharmacol. 1993, 44, 1201-1207), muscular dystrophy (Am. J. Pathol. 1986, 122, 193-198; 1987, 127, 461-466), myocardial infarction (J. Am. Coll. Cardiol. 1983, 2, 681-688), bacterial infection (Rev. Infect. Dis., 1983, 5, 5914-5921) and common cold (Biochem. 1995, 34, 8172-8179). The calcium-associated cysteine proteases calpains I and II have been associated with ischemia and hypoxia, Alzheimer's disease (Proc. Natl. Acad. Sci. U.S.A. 1993, 90, 2628-2632) and cataracts (J. Biol. Chem. 1993, 268, 1937-1940). These medical disorders are thought to be due, among other factors,to the deregulation of the above mentioned cysteine proteases class of enzymes. Therefore this class of enzymes is excellent targets for the development of specific inhibitors as possible therapeutic agents.

Several types of cysteine proteases inhibitors have been reported, such as peptide aldehydes (Biochim. Biophys. Acta 1991, 1073-43), nitriles (Biochim. Biophys.Acta 1990, 1035, 62-70), halomethyl ketones (Anal. Biochem. 1985, 149, 461-465; Acta. Biol. Med. Ger. 1981, 40, 1503-1511; Biochem. Phar. 1992, 44, 1201-1207), diazomethyl ketones (Biochem. J. 1988, 253, 751), acyloxy methyl ketones (J. Med. Chem. 1994, 37, 1833-1840; J. Am. Chem. Soc. 1988, 110, 4429-4431), ketomethylsulfonium salt (J. Biol. Chem. 1988, 263, 2768-2772), α-ketocarbonyl compounds (J. Med. Chem. 1993, 36, 3472-3480; 1994, 37, 2918-2929), vinyl sulfones (J. Med. Chem. 1995, 38, 3193-3196), monobactam derivatives (US patent Appln. S.N. 08/415055, 1995) and epoxysuccinyl derivatives (Agric. Biol. Chem. 1978, 42, 523-527). These inhibitors, in general, have a peptidyl

affinity group and a group r active towards the thiol of th cysteine residue in cystein prot as s. Some of them are clinically useful. However, the efficacy in vivo is not as much as expected on the basis of in vitro inhibitory activity and may be due to lower selectivity towards other proteases and poor pharmacokinetics. There exists a continuing need to develop new cysteine proteases inhibitors with high selectivity, lower toxicity and better pharmacokinetics.

In continuation of our efforts to find out the low molecular weight cysteine protease inhibitors for therapeutic uses, we have focused our attention at 6-substituted oxapenam derivatives on the basis of the molecular modeling studies of 3-substituted-4-oxa-1-azabicyclo [3,2,0] heptan-7-one derivatives.

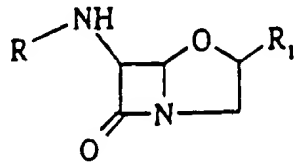
Summary of the invention

The present invention is based on the discovery that certain 6-substituted amino- 4-oxa-1-azabicyclo[3,2,0] heptan-7-one derivatives exhibit excellent cysteine protease inhibitory activity which may be used for treatment of different diseases such as muscular dystrophy, arthritis, myocardial infarction, Alzheimer's disease, bacterial infection, common cold, osteroporosis or cancer metastasis.

Our laboratory has been actively involved in the search for novel types of cysteine proteases inhibitors with high selectivity among the cysteine protease class of enzymes. We have reported that 3-substituted-4-oxa-1-azabicyclo [3,2,0] heptan-7-one derivatives exhibited good cysteine protease inhibitory activity. Further to optimize and enhance the activity, we studied the interaction of inhibitors with the papain and cathepsin B enzyme crystal structures. Molecular modeling studies suggested that the 1-N atom in 4-oxa-1-azabicyclo[3,2,0] heptan-7-one ring can be involved in hydrogen-bonding to a protonated Histidine in the active site of cysteine proteases. This binding may weaken the lactamic bond and activate the four membered ring towards acylation of the thiol of the cysteine residue in cystein proteases. A comparison of a model of a

substrate (Cbz (benzyloxycarbonyl)-Phe-Ala-Nme (NH-m thyl)) and that of the 4-oxa-1-azabicyclo[3,2,0] heptan-7-one ring in a tetrahedral intermediate complex with papain showed good superposition. We have also found that the substitution at position-6 of 4-oxa-1-azabicyclo[3,2,0] heptan-7-one will enhance the S2 subsite interaction with the papain enzyme. On the basis of this assumption, we have designed, synthesized and evaluated the cysteine protease inhibitory activity of various 6-substituted oxapenam and the finding is reported in the present invention.

In accordance to the present invention, there is provided 6-substituted amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one derivatives of general formula I or pharmaceutically acceptable salts thereof,



I

wherein:

R is a 1-2 amino acid residue wherein the amine thereof is unsubstituted or substituted with group R_2,

R_1 is (i) C1-C6 alkyl which is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy or amino, (ii) phenyl which is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy, amino or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy or amino, (iii) C1-C6 alkoxy which is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents ind p nd ntly selected from hydroxy,

halogen, cyano, carboxy or amino, or (iv) trifluoromethyl; and

R_2 is select d from the group consisting of hydrogen, $-COOR_3$, $-COR_4$ and $-SO_2R_5$, wherein

R_3 is C1-C6 alkyl which is unsubstituted or substituted with phenyl or heterocycle,

R_4 is selected from the group consisting of (i) C1-C6 alkyl which is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy or amino, (ii) C2-C4 alkenyl which is unsubstituted or substituted with heterocycle or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, amino or carboxy, (iii) C2-C4 alkynyl, (iv) C3-C6 cycloalkyl, (v) a phenyl group which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, trifluoromethyl and phenyl group which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino and trifluoromethyl, and (vi) a heterocycle which may be mono or bicyclic having 1-3 heteroatoms independently selected from N, S and O, and

R_5 is selected from the group consisting of (i) C1-C6 alkyl, (ii) alkenyl which is unsubstituted or substituted with heterocycle or phenyl, (iii) phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy group, trifluoromethyl and phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy group and trifluoromethyl, and (iv) naphthyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of

hydroxy, halogen, cyano, carboxy, amino, C1-C4 alkyl group, C1-C2 alkoxy group, trifluoromethyl and phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy group and trifluoromethyl.

The pharmaceutically acceptable salts of formula I are preferably selected from sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid salts.

The term "1-2 amino acid" used herein is one amino acid or one dipeptide consisting of two amino acids which are bound through a peptide bond. The term "1-2 amino acid" encompasses any of the "natural" amino acids and unnatural amino acids.

Examples of amino acids are α-amino acids which are the constituents of normal protein, or their optical isomers. Examples include glycine, D- or L-alanine, D- or L- valine, D- or L- leucine, D- or L-isoleucine, D- or L-serine, D- or L- threonine, D- or L- aspartic acid, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D- or L- lysine, D- or L-arginine, D- or L-phenylalanine, D- or L-tyrosine, D- or L-methionine, D- or L-proline and the like. Unnatural amino acids include, for example, D- or L-phenylglycine, D- or L-homophenylalanine, D- or L-pyridylalanine, D- or L-thienylalanine, D- or L-naphthylalanine, D- or L-halophenylalanine, D- or L-cyclohexylalanine, tert-butyl glycine and the like.

The term "heterocycle", unless otherwise described, used herein includes mono-, bi-, or tricyclic 5-14 membered rings having 1-4 heteroatoms selected from N, S and O. Examples of heterocycles are 1,2,3-triazole, 1,2,4-triazole, imidazole, pyrrole, pyrazole, thiophene, pyrrolidine, pyridine, piperidine, pyrimidine, piperazine, morpholine, thiomorpholine, 1-quinoline, 2-quinoline, isoalloxazine, phenoxazine, phenothiazine, and the like.

The 4-oxa-1-azabicyclo[3,2,0] heptan-7-one nucleus carries two asymmetric carbon atoms at position 5 and 6, and can therefore exist as

4-diastereoisomers. In general, the preferred isomer is that in which the hydrogen atoms at C5 and C6 are trans to each other this isomer has superior inhibitory activity against different cysteine proteases such as Cathepsin B, and Cathepsin L. Such diasterioisomers and their racemic mixtures are also included as cysteine protease inhibitors of the present invention.

More specifically, the most preferred embodiments of the present invention include the following compounds:

(5R,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-pronyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-isoleucyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-alanyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-leucyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-phenylglycyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one;

(5S,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one;

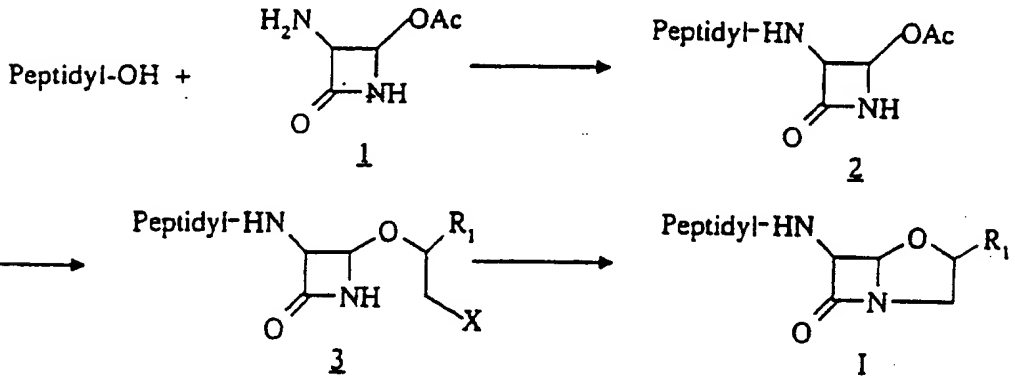
(5R,6S)-6-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-3-phenyl-4-oxa-1-azabicyclo[3,2,0] heptan-7-one; and

(5S,6S)-6-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-3-bromomethyl-4-oxa-1-azabicyclo[3,2,0] heptan-7-one.

Compounds of formula I may be utilized for treatment of different diseases, including muscular dystrophy, arthritis, myocardial infarction, Alzheimer's disease, bacterial infection, common cold, osteroporosis or cancer metastasis. Examples of cancer metastasis are breast, lung, liver, colon, brain and prostate cancers.

Description of Preferred Embodiments

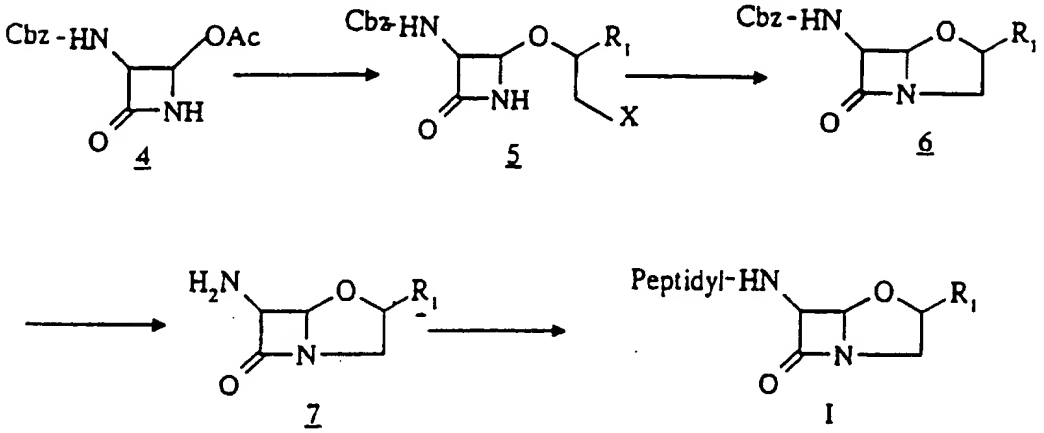
The present invention relates to the 6-substituted amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one derivatives having excellent cysteine protease inhibitory activity and selectivity among cysteine proteaess. The compounds of this invention are characterized by having a substitution at position 6 of 4-oxa-1-azabicyclo[3,2,0] heptan-7-one skeleton. The 6-substituted amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one derivatives were prepared by the general synthetic route as represented in scheme I.



The derivatives of general formula I were prepared from the common intermediate 1. The preparation of compound I was carried out by the synthetic route as described in Eur. J. Med. Chem. 1992, 27, 131-140 starting from 6-aminopenicillanic acid. The peptidyl group is a 1-2 amino acid residue as defined above with a protective group at N-terminal. The intermediate 1 was coupled either with protected peptidyl carboxylic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC), or with acid chloride in the presence of base, or with anhydride in the presence of base or activated ester, to produce compound 2. Compound 3 was obtained by reacting of 2 with 2-substituted ethanol in the presence of lewis acids such as zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, aluminium trichloride and the like, wherein X is a leaving group selected from a chlorine, bromine, iodine, methanesulfonyloxy or toluenesulfonyloxy group. Conv rsion of 3 to I was

done by cyclization using a suitable base such as potassium carbonate, sodium carbonate, cesium carbonate in a non reactive solvent.

Alternatively, the derivatives of general formula I were also prepared by the general synthetic route as represented in scheme II



The intermediate 4 was reacted with 2-substituted ethanol in the presence of lewis acids such as zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, aluminium trichloride and the like, wherein X is a leaving group selected from a chlorine, bromine, iodine, methanesulfonyloxy or toluenesulfonyloxy group to give compound 5. Cyclization of 5 using a suitable base such as potassium carbonate, sodium carbonate, cesium carbonate in a non reactive solvent gives 6-protected amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one 6. The benzyloxycarbonyl (denoted "Cbz") protected amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one 6 was deprotected by hydrogenation in the presence of a metal catalyst, such as Pd, Pt, or Rh, under normal pressure to high pressure to give compound 7. The derivatives of general formula I were obtained by reacting of amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one 7 with protected peptidyl carboxylic acid in the presence of DCC, or through acid chloride in the presence of base, or through anhydride in the presence of base or the activated ester.

In the above processes, the reactants are reacted together with solvent at elevated or low temp ratures for sufficient time to allow the

reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants, and would be readily understood by those of skill in the art.

Wherever a base is used in a reaction, it is preferably selected from the group consisting of triethyl amine, pyridine, 4-dimethylaminopyridine, diisopropylamine, 1,5-diazabicyclo [4,3,0] non-5-ene, 1,8-diazabicyclo [5,4,0] undec-7-ene, sodium carbonate, potassium carbonate and cesium carbonate. Preferred solvents for the reaction are non reactive solvents. Depending on the reactants, a solvent will generally be selected from the group consisting of benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, and the like. Solvent mixtures may also be utilized. Reaction temperatures generally range from between -70 °C to 150 °C. The preferred molar ratio of reactants are 1:1 to 5. The reaction time range from 0.5 to 72 hours, depending on the reactants.

The compounds of this invention, when used alone or in combination with other drugs as an agent for treating muscular dystrophy, arthritis, myocardial infarction, Alzheimer's disease, bacterial infection, common cold, osteroporosis or cancer metastasis in mammals including humans, may take pharmaceutical dosage forms including parenteral preparation such as injections, suppositories, aerosols and the like, oral preparations such as tablets, coated tablets, powders, granules, capsules, liquids and the like, and topical preparations such as lotions, solutions, creams, ointments or dusting powders. Injections are generally preferred. The above preparations are formulated in a manner known in the art.

For the formulation of solid preparations for oral administration, an excipient, and if desired, a binder, disintegrator, lubricant, coloring agent, corrigent, flavor, etc. is added to the compound of the invention, and then tablets, coated tablets, granules, powders, capsules or the like are prepared in a conventional manner.

For the formulation of injections, a pH adjusting agent, buffer, stabilizer, isotonic agent, local anesthetic or the like is added to the active ingredient of the invention. Injections for subcutaneus, intramuscular or intravenous administration can be prepared in the conventional manner.

For the formulation of suppositories, a base, and, if desired, a surfactant are added to the active ingredient of the invention, and the suppositories are prepared in a conventional manner.

The excipients useful for solid preparations for oral administration are those generally used in the art, such as lactose, sucrose, sodium chloride, starches, calcium carbonate, kaolin, crystalline cellulose, methyl cellulose, glycerin, sodium alginate, gum arabic and the like. Other ingredients which may be used in the formulations of the invention include binders such as polyvinyl alcohol, polyvinyl ether, polyvinyl pyrrolidone, ethyl cellulose, gum arabic, shellac, sucrose, water, ethanol, propanol, carboxymethyl cellulose, potassium phosphate and the like; lubricants such as magnesium stearate, talc and the like; and additives such as usual known coloring agents, disintegrators and the like. Examples of bases useful for the formulation of suppositories are oleaginous bases such as cacao butter, polyethylene glycol, lanolin, fatty acid triglycerides, witepsol (trademark, Dynamite Nobel Co. Ltd.) and the like. Liquid preparations may be in the form of aqueous or oleaginous suspensions, solutions, syrups, elixirs and the like, which can be prepared by a conventional way using additives.

For the formulation of topical preparations, the active ingredient of the invention can be incorporated into a cream, for example, comprising an aqueous emulsion of polyethylene glycols or liquid paraffin. The active ingredient of the invention can also be incorporated into an ointment comprising a white wax or white soft paraffin base together with such stabilizers and preservatives as may be required. Stabilizers and pres rvatives for topical preparations are well known to those of skill in the art.

The amount of the compound of formula I of the invention to be incorporated into the pharmaceutical composition of the invention varies with the dosage form, solubility and chemical properties of the compound, administration route, administration scheme and the like. Preferable the amount is about 1 to 25 w/w% in the case of oral preparations, about 0.1 to 5 w/w% in the case of injections which are parenteral preparations, and about 1 to 10 w/w% in the case of topical preparations.

The dosage of the compound I of the invention is suitably determined depending on the individual cases taking symptoms, age and sex of the subject and the like into consideration. Usually the dosage in the case of oral administration is about 50 to 1500 mg per day for an adult in 2 to 4 divided doses, and the dosage in the case of injection, for example, by intravenous administration is 2.0 ml (about 1 to 100 mg) which is administratered once a day for adults wherein the injection may be diluted with physiological saline or glucose injection liquid if so desired, and slowly administered over at least 5 minutes. The dosage in case of suppositories is about 1 to 1000 mg which is administered once or twice a day at an interval of 6 to 12 hours wherein the suppostories are administered by insertion into the rectum. For topical administration, the dosage is about 1 to 2500 mg which is administered one to four times a day.

Example 1

(5R,6S)-6-benzyloxycarbonylamino- 4-oxa-1-azabicyclo[3.2.0] heptan-7-one

A mixture of (3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (11.76 g, 42.3 mmole) which prepared by the known method (Eur. J. Med. Chem. 1992, 27, 131-140), 2-bromoethanol (3 ml, 42.3 mmole), and zinc acetate dihydrate (9.28 g, 42.3 mmole) in a mixture of benzene (100 ml) and toluene (100 ml) was refluxed for 5 hrs using Dean-Stark water separator. Aft r cooling, th reaction mixture was partitioned between ethyl acetate (800 ml), acetone (100 ml) and water (500 ml). Th

organic layer was washed with water, brine and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (6:4) as eluent and 2.04 g of (3S,4R)-3-benzyloxycarbonylamino-4-bromoethoxy-azetidin-2-one was obtained as white solid (yield: 14%).

A mixture of (3S,4R)-3-benzyloxycarbonylamino-4-bromoethoxy-azetidin-2-one (2.04 g, 5.945 mmole) and powder K_2CO_3 (903 mg, 6.54 mmole) in DMSO (20 ml) was stirred at room temperatute overnight and then diluted with ethyl acetate, washed with cold water, brine, and dried over sodium sulfate. After removal of the solvent, 1.43 g of the title compound was obtained.

Yield: 92 %

m.p. : 200 °C (dec.)

FAB-MS: 263 ($M+H^+$), calcd for $C_{13}H_{14}N_2O_4$ 262

IR (KBr, cm^{-1}) : 3295, 1782, 1711, 1652, 1513, 1440

^1H NMR ($CDCl_3$), d (ppm): 3.11 (1H, m), 3.81 (1H, m), 4.10 (2H, m), 5.12 (2H, s), 5.17 (1H, d, J=2.6), 5.29-5.38 (2H, m), 7.35 (5H, bs).

Example 2

(5R,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

(5R,6S)-6-benzyloxycarbonylamino- 4-oxa-1-azabicyclo[3,2,0] heptan-7-one (950 mg, 3.624 mmole) was hydrogenated with 3 g of 10 % palladium on activated carbon in ethyl acetate (80 ml) at 50 psi hydrogen pressure at room temperature for 6 hrs. after removal of catalyst by filtration, deprotected (5R,6S)-6-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one in ethyl acetate was obtained.

To a solution of N-benzyloxycarbonyl-L-phenylalanine (867 mg, 2.90 mmole) and triethylamine (323 mg, 3.2 mmole) in dichloromethane (20 ml), ethyl chloroformate (315 mg, 2.90 mmole) was added at -15 °C. The reaction mixture was stirred at -10 to 5 C for 1.5 hrs. Then a precooled (~ -15 C) solution of (5R,6S)-6-amino-4-oxa-1-azabicyclo[3,2,0]

heptan-7-one in ethyl acetate (see abov) was added at -15 °C and the resulting mixture was stirred at room temperature for 10 hrs. After removal of solvent, the residue was dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (55:45) as eluent and 205 mg of the title compound was obtained as white solid.

Yield: 17 %

m.p. : 144-145 °C

FAB-MS: 432 ($M+Na^+$), calcd for $C_{22}H_{23}N_3O_5$ 409

IR (KBr, cm^{-1}) : 3280, 1780, 1608, 1647, 1526, 1219

^1H NMR (DMSO-d_6), d (ppm): 2.67-3.14 (3H, m), 3.72 (1H, m), 4.05 (2H, m), 4.39 (1H, m), 4.93 (2H, s), 5.23 (1H, d, J=2.8), 5.42 (1H, dd, J=2.8, 9.1), 7.20-7.35 (10H, m), 7.58 (1H, d, J=8.8), 8.67 (1H, d, J=9.1).

Example 3

(5R,6S)-6-(N-benzyloxycarbonyl-L-prolyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one

By using a similar method described in example 2, the title compound was obtained by reacting N-benzyloxycarbonyl-L-proline with (5R,6S)-6-amino- 4-oxa-1-azabicyclo[3,2,0] heptan-7-one.

Yield: 5 %

FAB-MS: 360 ($M+H^+$), calcd for $C_{18}H_{21}N_3O_5$ 359

IR (KBr, cm^{-1}) : 3295, 2930, 1694, 1653, 1522, 1409, 1346

^1H NMR ($CDCl_3$), d (ppm): 1.91-2.38 (4H, m), 3.53 (2H, bs), 3.81 (1H, m), 4.05 (2H, m), 4.37 (1H, bs), 5.16 (3H, m), 5.50 (1H, dd, J=2.8, 9.2), 6.50 (1H, bs), 7.35 (5H, bs).

Example 4

(5R,6S)-6-(N-benzyloxycarbonyl-L-isoleucyl)-amino-4-oxa-1-azabicyclo[3,2,0]heptan-7-one

By using a similar method described in example 2, the title compound was obtained by reacting N-benzyloxycarbonyl-L-isoleucine with

(5R,6S)-6-amino- 4-oxa-1-azabicyclo[3,2,0] heptan-7-on .

Yield: 5 %

m.p. : 100 °C (dec.)

FAB-MS: 376 ($M+H^+$), calcd for $C_{19}H_{25}N_3O_5$ 375

IR (KBr, cm^{-1}) : 3310, 2935, 1700, 1651, 1515, 1448

^1H NMR ($CDCl_3$), d (ppm): 0.86-0.97 (6H, m), 1.03-1.26 (2H, m), 1.47 (1H, m), 3.06-3.18 (1H, m), 3.74-3.86 (1H, m), 3.98-4.17 (3H, m), 5.10 (2H, s), 5.16 (1H, d, J=2.8), 5.32 (1H, d, J=9.6), 5.50 (1H, dd, J=2.8, 9.6), 6.49 (1H, d, J=9.1), 7.35 (5H, m).

Example 5

(5R,6S)-6-(N-benzyloxycarbonyl-L-alanyl)-amino-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

(3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (2.00 g, 7.188 mmole) was hydrogenated with 2 g of 10 % palladium on activated carbon in ethyl acetate (50 ml) at 50 psi hydrogen pressure at room temperature for 1.5 hrs. After removal of catalyst by filtration, the deproteced (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate was obtained.

To a solution of N-benzyloxycarbonyl-L-alanine (1.604 g, 7.188 mmole) and triethylamine (799 mg, 7.91 mmole) in dichloromethane (30 ml), ethyl chloroformate (738 mg, 6.83 mmole) was added at -15 °C. The reaction mixture was stirred at -10 to 5 °C for 1.5 hrs. Then a precooled (ca. -15 °C) solution of (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate (see above) was added at -15 °C and the resulting mixture was stirred at 0 °C to room temperature for 2 hrs. After removal of solvent, the residue was dissolved in ethyl acetate, washed with cold saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue was recrystallized from dichloromethane and (3S,4S)-3-(N-benzyloxycarbonyl-L-alanyl) amino-4-acetoxy-azetidin-2-on was obtained (1.36 g, 54 % yield).

A mixture of (3S,4S)-3-(N-benzyloxycarbonyl-L-alanyl)amino-4-

acetoxy-azetidin-2-one (1.36 g, 3.894 mmole), 2-bromoethanol (440 mg, 3.5 mmole), and zinc acetate dihydrate (642 mg, 2.9 mmole) in a mixture of benzene (40 ml) and toluene (40 ml) was refluxed for 5 hrs using Dean-Stark water separator. After cooling, the reaction mixture was partitioned between ethyl acetate (200 ml), acetone (25 ml) and water (150 ml). The organic layer was washed with water, brine and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (2:1) as eluent and (3S,4R)-3-(N-benzyloxycarbonyl-L-alanyl)amino-4-bromoethoxy-azetidin-2-one was obtained (169 mg, 12 % yield).

A mixture of (3S,4R)-3-(N-benzyloxycarbonyl-L-alanyl)amino-4-bromoethoxy-azetidin-2-one (159 mg, 0.384 mmole) and powder K_2CO_3 (58 mg, 0.42 mmole) in DMSO (2 ml) was stirred at room temperatute overnight and then diluted with ethyl acetate, washed with cold water, brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (3:1) as eluent and 49 mg of the title compound was obtained.

Yield: 38 %

m.p. : 150 °C (dec.)

FAB-MS: 356 ($M+Na^+$), calcd for $C_{16}H_{19}N_3O_5$ 333

IR (KBr, cm^{-1}) : 3285, 2950, 1701, 1679, 1645, 1527, 1444, 1325

^1H NMR ($CDCl_3$), d (ppm): 1.39 (3H, d, J=7.0), 3.12 (1H, m), 3.83 (1H, m), 4.08 (2H, m), 4.25 (1H, m), 5.11 (2H, s), 5.16 (1H, d, J=2.8), 5.20 (1H, bs), 5.49 (1H, dd, J=2.8, 9.3), 6.56 (1H, bs), 7.34 (5H, m).

Example 6

(5R,6S)-6-(N-benzyloxycarbonyl-L-leucyl)-amino-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

By using a similar method described in example 5, the title compound was synthesized from (3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-on and

N-benzyloxycarbonyl-L-leucine.

Yield: 1.5 % (total yield for 3 steps)

m.p. : 90 °C (dec.)

FAB-MS: 398 ($M+Na^+$), calcd for $C_{19}H_{25}N_3O_5$ 375

IR (KBr, cm^{-1}) : 3285, 1780, 1678, 1645, 1526, 1444, 1325

^1H NMR (DMSO-d_6), d (ppm): 0.86 (6H, m), 1.17-1.62 (3H, m), 3.05 (1H, m), 3.71 (1H, m), 4.06 (2H, m), 4.17 (1H, m), 5.02 (2H, s), 5.19 (1H, d, J=2.8), 5.35 (1H, dd, J=2.8, 9.0), 7.35 (5H, m), 7.44 (1H, d, J=8.5), 8.48 (1H, d, J=9.0).

Example 7

(5R,6S)-6-(N-benzyloxycarbonyl-phenylglycyl)-amino-4-oxa-1-azabicyclo[3.2.0]heptan-7-one

By using a similar method described in example 5, the title compound was synthesized from (3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one and

N-benzyloxycarbonyl-phenylglycine.

Yield: 0.7 % (total yield for 3 steps)

m.p. : 119-120 °C

FAB-MS: 418 ($M+Na^+$), calcd for $C_{21}H_{21}N_3O_5$ 395

IR (KBr, cm^{-1}) : 3295, 2925, 1782, 1663, 1518

^1H NMR ($CDCl_3$), d (ppm): 3.10 (1H, m), 3.76 (1H, m), 4.04 (2H, m), 5.09 (2H, m), 5.16 (1H, d, J=2.8), 5.25 (1H, bs), 5.46 (1H, dd, J=2.9, 9.0), 5.96 (1H, bs), 6.22 (1H, d, J=9.0), 7.36 (10H, bs).

Example 8

(5S,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

(3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (6.00 g, 21.6 mmole) was hydrogenated with 6 g of 10 % palladium on activated carbon in ethyl acetate (150 ml) at 50 psi hydrogen pressure at room temperatur for 1.5 hrs. after removal of catalyst by filtration, the deproteced (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate was

obtained.

To a solution of N-benzyloxycarbonyl-L-ph nylalanine (6.78 g, 22.6 mmole) and 1-hydroxybenzotriazole (3.05 g, 22.6 mmole) in tetrahydrofurane (150 ml), dicyclohexylcarbodiimide (4.45 g, 21.6 mmole) was added at 0 °C. The reaction mixture was stirred at room temperature for 1.5 hrs and then cooled with an ice bath. The resulting N,N'-dicyclohexylurea was removed by filtration. Then a precooled (ca. -15 °C) solution of (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate (see above) was added at -15 °C and the resulting mixture was stirred at 0 °C to room temperature for 2 hrs. After removal of solvent, the residue was dissolved in ethyl acetate, washed with cold saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue was recrystallization from ethyl acetate-hexane and (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-acetoxy-azetidin-2-one was obtained (4.57 g, 50 % yield).

A mixture of (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-acetoxy-azetidin-2-one (4.57 g, 10.8 mmole), 2-bromoethanol (1479 mg, 11.83 mmole), and zinc acetate dihydrate (1.78 g, 8.128 mmole) in a mixture of benzene (150 ml) and toluene (150 ml) was refluxed for 7 hrs using Dean-Stark water separator. After cooling, the reaction mixture was partitioned between ethyl acetate (1000 ml), acetone (100 ml) and water (500 ml). The organic layer was washed with water, brine and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (55:45) as eluent and (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-bromoethoxy-azetidin-2-one was obtained (650 mg, 12 % yield).

A mixture of (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)amino-4-bromoethoxy-azetidin-2-one (1.43 g, 2.918 mmole) and powder K_2CO_3 (444 mg, 3.2 mmole) in DMSO (20 ml) was stirred at room temperatute overnight and then dilut d with ethyl acetat , washed with cold water, brine, and dried over sodium sulfate. Aft r removal of th solvent, the

residue was recrystallized from ethyl acetate-hexane and 590 mg of the title compound was obtain d.

Yield: 49 %

m.p. : 175-176 °C

FAB-MS: 432 ($M+Na^+$), calcd for $C_{22}H_{23}N_3O_5$ 409

IR (KBr, cm^{-1}) : 3285, 1779, 1683, 1659, 1525

^1H NMR (DMSO-d_6), d (ppm): 2.72-2.97 (3H, m), 3.76 (1H, m), 4.00 (2H, m), 4.23 (1H, m), 4.53 (1H, d, J=7.7), 4.96 (2H, d, J=2.3), 5.05 (1H, s), 7.27 (10H, bs), 7.62 (1H, d, J=8.5), 8.95 (1H, d, J=7.7).

Example 9

(5R,6S)-6-[N-(3-phenylpropionoyl)-L-phenylalanyl]-amino-3-phenyl-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

A mixture of 1-phenyl-1,2-ethanediol (1.38 g, 10 mmole), immidazole (817 mg, 12 mmole), and tert-butylchlorodimethylsilane (1.81 g, 12 mmole) in DMF 15 (ml) was stirred at 0 °C for 1.5 hrs and then at room temperature overnight. The resulting mixture was diluted with ethyl acetate, washed with water, brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (1:4) as eluent. 2-(tert-butyldimethylsilyl)oxy-1-phenylethanol was obtained as an oil (2.6 g, 100 % yield).

1H NMR (CDCl3), d (ppm): 0 (6H, s), 0.85 (9H, s), 2.92 (1H, d, J=2.1), 3.45-3.75 (2H, m), 4.60-4.75 (2H, m), 7.20-7.35 (5H).

(3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (5.56 g, 20 mmole) was hydrogenated with 5.6 g of 10 % palladium on activated carbon in ethyl acetate (120 ml) at 50 psi hydrogen pressure at room temperature for 1.5 hrs. After removal of catalyst by filtration, the deproteced (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate was obtained.

To a solution of N-(3-phenylpropionoyl)-L-phenylalanine (5.95 g, 20 mmol) and 1-hydroxybenzotriazole (2.7 g, 20 mmole) in tetrahydrofurane

(150 ml), dicyclohexylcarbodiimide (4.12 g, 21.6 mmole)/THF (50 ml) was added at 0 °C. The reaction mixture was stirred at room temperature for 1.5 hrs and then cooled with an ice bath. The resulting N,N'-dicyclohexylurea was removed by filtration. Then a precooled (ca. -15 °C) solution of (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate (see above) was added at -15 °C and the resulting mixture was stirred at 0 °C to room temperature for 2 hrs. After removal of solvent, the residue was dissolved in ethyl acetate, washed with cold saturated $NaHCO_3$ solution, water, brine and dried over sodium sulfate. After removal of solvent, the residue was recrystallized from ethyl acetate and (3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-acetoxy-azetidin-2-one was obtained (7.2 g, 85 % yield).

1H NMR (DMSO-d_6), d (ppm): 2.09 (3H, s), 2.36 (2H, m), 2.68 (2H, m), 2.75 (1H, dd, J=14, 10), 3.01 (1H, dd, J=14, 5), 4.53 (1H, m), 4.60 (1H, dd, J=8, 1), 5.75 (1H, d, J=1), 7.05-7.30 (10H, m), 8.15 (1H, d, J=8), 8.72 (1H, d, J=8), 9.17 (1H, s).

A mixture of (3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-acetoxy-azetidin-2-one (4.36 g, 10.30 mmole), 2-(tert-butyldimethylsilyl)oxy-1-phenylethanol (2.6 g, 10.30 mmole), and zinc acetate dihydrate (2.26 g, 10.30 mmole) in a mixture of benzene (70 ml) and toluene (70 ml) was refluxed overnight using Dean-Stark water separator. After cooling, the reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water, brine and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (4:3) as eluent and (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-{2-(tert-butyldimethylsilyl)oxy-1-phenyl ethoxy}-azetidin-2-one was obtained (440 mg, 7 % yield).

^{1}H NMR (DMSO-d_6), d (ppm): 0 (6H, s), 0.83 (9H, s), 2.25-2.40 (2H, m), 2.65-3.10 (4H, m), 3.80-4.00 (2H, m), 4.45-4.55 (2H, m), 5.00-5.15 (2H, m), 7.00-7.40 (15H, m), 7.85 (1H, s), 8.30 (1H, m), 8.65 (1H, s).

A THF solution of 1N Bu_4NF (0.84 ml, 0.84 mmole) containing AcOH (35 mg, 0.56 mmole) was added to a solution of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-{2-(tert-butyldimethylsilyl)oxy-1-phenylethoxy}-azetidin-2-one (430 mg, 0.70 mmole) in THF (5 ml) at 0-5 °C. The mixture was stirred at room temperature for 3 hrs, then poured into a silica gel column. The column was eluented with methanol-ethyl acetate (5:95) and 260 mg of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-(2-hydroxy-1-phenylethoxy)-azetidin-2-one was obtained (74 % yield).

^1H NMR (DMSO-d_6), d (ppm): 2.30-2.45 (2H, m), 2.75-3.10 (4H, m), 3.75-4.10 (3H, m), 4.80-5.05 (2H, m), 6.40-6.70 (2H, m), 7.0-7.40 (15H, m), 7.61 (1H, bs), 7.95 (1H, d, J=8), 8.35 (1H, d, J=8).

p-Toluenesulfonyl chloride (119 mg, 0.62 mmol) was added to an ice-cooled solution of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-(2-hydroxy-1-phenylethoxy)-azetidin-2-one (260 mg, 0.52 mmol) and pyridine (493 mg, 6.2 mmol) in dichloromethane (7 ml). The mixture was stirred at 0 °C for 2 hrs and then at room temperature overnight. After removal of solvent, the residue was purified by silica gel column chromatography using ethyl acetate-hexane (8:3) as eluent and 160 mg of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-{2-(p-toluenesulfonyl)oxy-1-phenylethoxy}-azetidin-2-one was obtained (47 % yield).

^1H NMR (CDCl3), d (ppm): 2.30-2.45 (5H, m), 2.75-3.05 (4H, m), 4.20-4.40 (2H, m), 4.95-5.15 (2H, m), 6.40-6.60 (2H, m), 7.0-7.4 (18H, m), 7.75 (2H, dd, J=8.3, 3), 8.15 (1H, d, J=1.2), 8.55 (1H, d, J=7.6).

A mixture of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-{2-(p-toluenesulfonyl)oxy-1-phenylethoxy}-azetidin-2-one (160 mg, 0.244 mmol), lithium bromide (133 mg, 1.525 mmol) and HMPA (4 ml) was stirred at 60 °C for 1.5 hrs. The resulting mixture was diluted with ethyl acetate , washed with water, brine, and dried over sodium sulfate. After removal of solvent, the residue was purified by silica gel

column chromatography using hexane-ethyl acetate (1:2) as eluent and 100 mg (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-(2-bromo-1-phenylethoxy)-azetidin-2-one as white foam was obtained in 72% yield.

A mixture of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-(2-bromo-1-phenylethoxy)-azetidin-2-one (100 mg, 0.177 mmole) and powder K_2CO_3 (27 mg, 0.195 mmole) in DMSO (3 ml) was stirred at room temperatute overnight and then diluted with ethyl acetate, washed with cold water, brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:2) as eluent, gave the title compound.

Yield: 30 mg (35%)

m.p. : 132-135°C

IR (KBr, cm^{-1}) : 3450, 3275, 1785, 1656, 1513,1414, 1179, 693;

^1H NMR ($CDCl_3$), d (ppm): 2.39(2H, m), 2.80-3.03(4H, m), 3.69(1H, d, J=6.1Hz), 3.82(1H, d, J=61Hz), 4.83(1H, d, J=3.5Hz), 5.15(1H, abq, J=8.8, 3.5), 5.11(1H, m), 6.17-6.43(1H, m), 7.01-7.57(15H, m), 8.22(1H,s), 8.44-8.66(1H, m)

Example 10

(5S,6S)-6-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-3-bromomethyl-4-oxa-1-azabicyclo[3.2.0] heptan-7-one

A mixture of (3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-acetoxy-azetidin-2-one (1.67 g, 3.95 mmole), 1,3-dibromo-2-propanol (689 mg, 3.16 mmole), and zinc acetate dihydrate (435 mg, 1.98 mmole) in a mixture of benzene (25 ml) and toluene (25 ml) was refluxed overnight using Dean-Stark water separator. After cooling, the reaction mixture was partitioned between ethyl acetate (200 ml), acetone (40 ml) and water (150 ml). The organic layer was washed with water, brine and dried over sodium sulfate. Aft r removal of the solvent, the residue was purified by silica gel column chromatography using thyl

acetate-h xan (4:3) as eluent and (3S,4S)-3-{N-(3-phenylpropionoyl)-L-ph nylalanyl}amino-4-(1,3-dibromoprop-2-yl)oxy-az tidin-2-on was obtained (170 mg, 7 % yield).

^{1}H NMR (CDCl3), d (ppm): 2.35-2.55 (2H, m), 2.75-3.10 (4H, m), 3.40-3.55 (4H, bs), 3.80-3.95 (1H, m), 4.39 (1H, d, J=7), 4.75-4.90 (1H, m), 5.09 (1H, s), 6.80 (1H, d, J=8), 7.0-7.30 (10 H, m), 7.66 (1H, s), 7.83 (1H, d, J=7).

A mixture of (3S,4R)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}amino-4-(1,3-dibromoprop-2-yl)oxy-azetidin-2-one (170 mg, 0.29 mmole) and powder K_2CO_3 (44.5 mg, 0.322 mmole) in DMSO (3 ml) was stirred at room temperatute overnight and then diluted with ethyl acetate, washed with cold water, brine, and dried over sodium sulfate. After removal of the solvent, the residue was purified by silica gel preparative plate using hexane-ethyl acetate (1:2) as solvent for developing the plates. Out of 4 fractions, fraction 2 was obtained as title compound.

Yield: 20 mg (14%)

m.p. : 150-152°C

IR (KBr, cm^{-1}) : 3405, 1773, 1640, 1529, 1225;

^{1}H NMR (DMSO-d_6), d (ppm): 2.46(2H, t), 2.85-3.00(6H, m), 3.35-3.44(2H, m), 4.06(1H, m), 4.43(2H, m), 4.77(1H, m), 5.23(1H, s), 6.32(1H, d, J=8.1Hz), 7.08-7.26(11H, m)

Testing of inhibitors for inhibition of Cathepsin B and L

Test Example 1

In vitro assay procedure for cathepsin B

The compounds of formula I were tested for inhibition of cathepsin B using the known method (A.J. Barret et al., Biochem. J. 1982, 201, 189-198). To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin B, diluted to give approximate 10 F units/min, buffer: 56 mM sodium acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20

µl of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. R ading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Test Example 2

In vitro assay procedure for cathepsin L

To a 170 µl of enzyme-buffer mixture (enzyme: r rat cathepsin L, diluted to give approximate 15 F units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) a 10 µL of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, a 20 µl of 1 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the fluoroscan reader (excitation at 380 nm emission at 460 nm).

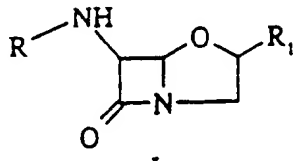
A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC_{50} is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Table 1 In vitro inhibitory activity of compounds of formula I on cysteine proteases

Example No.	IC_{50} (μM)	
	Cathepsin B	Cathepsin L
1	>50	>50
2	4.56	0.26
3	>50	9.40
4	>50	9.88
5	>50	38
6	30	0.60
7	>50	1.83
8	12.2	0.004
9	>50	>50
10	1.91	0.016

We Claim:

1. A 6-substituted amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one compound of formula I, or a pharmaceutically acceptable salt thereof or diastereoisomer thereof,



I

wherein:

R is a 1-2 amino acid residue wherein the amine thereof is unsubstituted or substituted with group R_2,

R_1 is selected from the group consisting of (i) C1-C6 alkyl which is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy and amino, (ii) phenyl which is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy, amino and phenyl which is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy and amino, (iii) C1-C6 alkoxy which is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from hydroxy, halogen, cyano, carboxy and amino, and (iv) trifluoromethyl; and

R_2 is selected from the group consisting of hydrogen, $-COOR_3$, $-COR_4$ and $-SO_2R_5$, wherein

R_3 is C1-C6 alkyl which is unsubstituted or substituted with phenyl or het rocycle,

R_4 is selected from the group consisting of (i) C1-C6 alkyl which is unsubstitut d or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, carboxy and amino, (ii) C2-C4 alkenyl which is unsubstituted or substituted with heterocycle or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents independently selected from the group consisting of hydroxy, halogen, cyano, amino and carboxy, (iii) C2-C4 alkynyl, (iv) C3-C6 cycloalkyl, (v) a phenyl group which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, trifluoromethyl and phenyl group which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino and trifluoromethyl, and (vi) a heterocycle which may be mono or bicyclic having 1-3 heteroatoms independently selected from the group consisting of N, S and O, and

R_5 is selected from the group consisting of (i) C1-C6 alkyl, (ii) alkenyl which is unsubstituted or substituted with heterocycle or phenyl, (iii) phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy group, trifluoromethyl and phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy group and trifluoromethyl and (iv) naphthyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, cyano, carboxy, amino, C1-C4 alkyl group, C1-C2 alkoxy group, trifluoromethyl and phenyl which is unsubstituted or substituted by 1-3 substituents independently selected from the group consisting of hydroxy, halogen, carboxy, cyano, amino, C1-C4 alkyl group, C1-C2 alkoxy

group and trifluoromethyl.

2. The compound of claim 1, wherein the amino acids comprise natural amino acids.

3. The compound of claim 1, wherein the amino acids are selected from the group consisting of glycine, D- or L-alanine, D- or L- valine, D- or L- leucine, D- or L- isoleucine, D- or L-serine, D- or L- threonine, D- or L-aspartic acid, D- or L-glutamic acid, D-or L-asparagine, D- or L-glutamine, D- or L- lysine, D- or L-arginine, D- or L-phenylalanine, D- or L-phenylglycine, D- or L-tyrosine, D- or L-methionine and D- or L-proline.

4. The compound of claim 1, wherein the hydrogen atoms at the two asymmetric carbon atoms at positions 5 and 6 are trans to each other.

5. The compound of claim 1, wherein the compound is selected from the group consisting of:

(5R,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-prolyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-isoleucyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-alanyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-L-leucyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-(N-benzyloxycarbonyl-phenylglycyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5S,6S)-6-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-oxa-1-azabicyclo[3,2,0] heptan-7-one;

(5R,6S)-6-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-3-phenyl-4-oxa-1-azabicyclo[3,2,0] heptan-7-one; and

(5S,6S)-6-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-3-bromo methyl -4-oxa-1-azabicyclo[3,2,0] heptan-7-one.

6. A pharmaceutical composition suitable for the treatment of a condition selected from the group consisting of muscular dystrophy, arthritis, myocardial infarction, Alzheimer's disease, bacterial infection, common cold, osteroporosis and cancer metastasis, comprising a condition-treating effective amount of a compound of claim 1 in combination with a pharmaceutically acceptable carrier or diluent.

7. The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is an oral pharmaceutical composition, and the compound is present in the pharmaceutical composition in an amount of about 1 to 25 w/w%.

8. The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is a parenteral pharmaceutical composition, and the compound is present in the pharmaceutical composition in an amount of about 0.1 to 5 w/w%.

9. The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is a topical pharmaceutical composition, and the compound is present in the pharmaceutical composition in an amount of about 1 to 10 w/w%.

10. A method of inhibiting cysteine protease in a patient in need of such inhibition, comprising administering to the patient a cysteine-protease inhibiting effective amount of a compound of claim 1.

11. The method of claim 10, wherein the compound is orally administered in a daily dose of about 50 to 1500 mg.

12. The method of claim 11, wherein the daily dose is divided into 2 to 4 individual doses.

13. The method of claim 10, wherein the compound is injectably administered in a daily dose of about 1 to 100 mg.

14. The method of claim 13, wherein the compound is slowly administered over a time period of at least 5 minutes.

15. Th method of claim 10, wherein the compound is rectally administer d in a daily dose of about 1 to 1000 mg.

16. The method of claim 15, wherein the daily dose is divided into 1 to 2 individual doses.

17. The method of claim 10, wherein the compound is topically administered in a daily dose of about 1 to 2500 mg.

18. The method of claim 17, wherein the daily dose is divided into 1 to 4 individual doses.

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/IB 97/00382

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 C07K5/08 C07D503/00 A61K38/55 A61K31/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 18807 A (TAIHO PHARMACEUTICAL CO.) 13 July 1995 see claims ---	1-18
A	DE 34 27 651 A (CIBA-GEIGY AG) 27 June 1985 see claims ---	1-18
A	US 4 202 819 A (M. KELLETT ET AL.) 13 May 1980 see the whole document ---	1-18
A	WO 88 10266 A (GRUBB A.) 29 December 1988 see claims ---	1-18

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
10 June 1997	18.06.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Chouly, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In. .tional Application No
PCT/IB 97/00382

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 96 32408 A (SYNPHAR LAB.,INC.) 17 October 1996 cited in the application see claims -----	1-18

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 97/00382

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 10-18
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort jusufying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. .tional Application No
PCT/IB 97/00382

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9518807 A	13-07-95	AU 1325095 A	01-08-95
		CA 2157602 A	13-07-95
		EP 0688324 A	27-12-95
		JP 8507552 T	13-08-96
		ZA 9500106 A	07-02-96
DE 3427651 A	27-06-85	NONE	
US 4202819 A	13-05-80	AT 4212 T	15-08-83
		EP 0019148 A	26-11-80
		JP 55149287 A	20-11-80
WO 8810266 A	29-12-88	AU 604944 B	03-01-91
		AU 1960488 A	19-01-89
		EP 0418233 A	27-03-91
		JP 2503917 T	15-11-90
		US 5037957 A	06-08-91
WO 9632408 A	17-10-96	AU 4951896 A	30-10-96